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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/081,118	02/25/2002	Didier Lefevre	20198/0059	8544

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EXAMINER	
GABEL, GAILENE	
ART UNIT	PAPER NUMBER
1641	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/31/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/081,118	LEFEVRE ET AL.	
	Examiner	Art Unit	
	Gailene R. Gabel	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 December 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3-6,9-11 and 19-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-6,9-11 and 19-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 18, 2006 has been entered.

Amendment Entry

2. Applicant's amendment and arguments, filed on December 18, 2006, is acknowledged and has been entered. Claims 19, 20, and 23 have been amended. Currently, claims 3-6, 9-11, and 19-25 are pending. Claims 3-6, 9-11, and 19-25 are under examination.

Withdrawn Rejections

3. All rejections not reiterated herein, have been withdrawn.

4. In light of Applicant's amendment and arguments, the rejection of claims 22-25 under 35 U.S.C. 112, first paragraph, as containing new matter which was not described in the specification, is hereby, withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 3-6, 11, and 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sakata (US 5,496,734) in view of Haas et al. (Cation-Anion Cotransport, Methods in Enzymology, 173: 280-91 (1989)), and if necessary, in light of Applicant's French Patent no. 97 01090 (31 January 1997).

Sakata discloses a reagent comprising a lysing agent and a fluorescent stain. The lysing reagent comprises non-ionic detergent such as quaternary ammonium salts which may be present at a concentration that lyses erythrocytes. The fluorescent stain may be any one of Thiazole Orange, Acridine Orange, ethidium bromide, and propidium bromide. Thiazole Orange, Acridine Orange, ethidium bromide, and propidium bromide have inherent capability to permeate through permeabilized cell membrane of nucleated unlysed cells such as leucocytes, so as to label intracellular nucleic acids (see column 6, line 64 to column 7, line 7; column 8; lines 1-28 and lines 44-54). The reagent further includes non-ionic surfactants (polyoxyethylene sorbitans and ethers), anticoagulant, buffer, and alcohol (see column 9, lines 1-5 and 14-44; and column 10, lines 10-44). The nonionic surfactants is used at a concentration that does not destroy the whole cell

membrane of nucleated cells such as leucocytes but is sufficient to damage their cell membrane so as to make it permeable, and also sufficient to lyse erythrocytes so make measurement of leucocytes easy (see column 7, lines 48-67). The reagent may also include at least one ionic surfactant which functions to remove constituents of leucocytic cell membrane; thus yielding pores to allow passage of substances such as stain into the cell and also assists in the lysis of erythrocytes (see column 9, lines 50-65 and column 10, line 63 to column 11, line 8). Sakata et al. provide that fixing agents such as formaldehyde and gluteraldehyde are used in prior art (see column 2, lines 12-19). Sakata et al. uses the reagent for identifying, counting, and classifying leucocytes in anticoagulant treated whole blood sample without removing erythrocytes (see column 6, lines 53-63).

Sakata et al. differ from the instant invention in failing to teach incorporation of ionophore into a reagent for identifying and counting nucleated blood cells.

Haas et al. provide use of valinomycin in cation-anion cotransport, as an ionophore to demonstrate different cell membrane permeability of cells to specific elements, i.e. sodium and potassium. Haas et al. specifically provide use of valinomycin as ionophore in conjunction with reagents or methods that employ potential sensitive dyes. Haas et al. show investigations of valinomycin with different cells and successfully demonstrated that there is electroneutrality of (Na+ + K+ + 2Cl-) cotransport in MDCK cells.

Applicant admits, by way of disclosure at page 4, lines 23-30, that inclusion of ionophores in a reagent solution assists in penetration of specific molecules into cells

(selectively increases permeability of potential-sensitive molecules into cell membrane of blood cells as provided by Haas) and mentions such concept in Applicant's French Patent no. 97 01090, dated 31 January 1997. Accordingly, ionophores are known to inherently increase permeability of cell membranes to specific selected molecules.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate valinomycin as an ionophore as taught by Haas into the reagent taught by Sakata for use in identifying, counting, and classifying blood cells, because Haas specifically provided that ionophores such as antibiotics (valinomycin) are capable of increasing permeability of cell membrane in blood cells to charge-sensitive molecules, such characteristic being a known inherent property of ionophores in reagents, and such enhancement in hematological compositions having potential sensitive dyes including that taught and used by Sakata, allows for more accurate detection, identification, and classification of nucleated blood cells in a sample because it enhances penetration of specific stains and other molecules of the reagent into intracellular blood cell environment.

6. Claims 9, 10, and 22-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sakata (US 5,496,734) in view of Haas et al. (Cation-Anion Cotransport, Methods in Enzymology, 173: 280-91 (1989)), and if necessary, in light of Applicant's French Patent no. 97 01090 (31 January 1997) as applied to claims 3-6, 11, and 19-21, and further in view of Ledis et al. (US Patent 4,751,179).

Sakata, Haas et al., and also Applicant's French Patent no. 97 01090 are discussed supra. Sakata, Haas et al., and also Applicant's French Patent no. 97 01090 differ from the instant invention in failing to teach incorporating saponins and also a membrane fixing agent into the reagent.

Ledis et al. disclose a reagent system having mixtures of saponins and other ionic or non-ionic detergents into hematological reagent to lyse red blood cells. Ledis et al. also disclose incorporation of glutaraldehyde as a mixing agent into the reagent system. According to Ledis et al., the reagent system is used to causatively treat whole blood in order to stromatolyze anucleated red blood cells and modify nucleated leucocytes or white blood cells so as to differentially define and identify distinct clusters or populations via parameters including light scatter and fluorescence intensity. See Abstract.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate saponins with other detergents as taught by Ledis into the reagent and method taught by Sakata and modified by Haas because saponins in mixtures with other lysing agents are obvious variations of lysing detergent mixtures known and commonly used in the hematological art for treating whole blood samples and removing interfering effects by red blood cells.

In as far as the concentration range of membrane fixing reagent used in the reagent, it is maintained that the amount of elements or components for incorporation into a reagent, are all result effective variables which the prior art references have shown may be altered in order to achieve optimum results. It has long been settled to

be no more than routine experimentation for one of ordinary skill in the art to discover an optimum value of a result effective variable. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum of workable ranges by routine experimentation." Application of Aller, 220 F.2d 454, 456, 105 USPQ 233, 235-236 (C.C.P.A. 1955). "No invention is involved in discovering optimum ranges of a process by routine experimentation." Id. at 458, 105 USPQ at 236-237. The "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." Application of Boesch, 617 F.2d 272, 276, 205 USPQ 215, 218-219 (C.C.P.A. 1980). Since Applicant has not disclosed that the specific limitation recited in instant claim 23 is for any particular purpose or solve any stated problem, and various matrices, solutions and parameters appear to work equally as well, absent unexpected results, it would have been obvious for one of ordinary skill to discover the optimum workable ranges of the reagents and methods disclosed by the prior art by normal optimization procedures.

Response to Arguments

7. Applicant's arguments filed on December 18, 2006 have been considered but are not persuasive.

A) Applicant argues that the combination of Sakata et al. with Haas et al. does not render obvious the claimed invention because the Sakata et al. reference differs not only in failing to disclose use of ionophore but also its use of a “pretreatment method”.

In response, the pretreatment method as taught by Sakata et al. comprises contacting a whole blood sample with a reagent consonant or otherwise, rendered obvious (by Haas et al. and in light of Veriac et al.) to the reagent and its components recited in claims 19 and 20. Alternatively, if the reagent of the claimed invention were to be used in an assay, i.e. such as for identifying and counting nucleated cells in a sample having erythrocytes, then it appears that the Applicant's sample having erythrocytes need be contacted; hence, pretreated, with the reagent of the claimed invention.

In as far as the recitation of the reagents intended use, i.e. simultaneous lysis, fixation, and staining of intracellular material, the recitation of intended use of the claimed invention must result in a structural difference between the claimed invention and the combined prior art in order to patentably distinguish the claimed invention from the prior art. If the prior art structure is capable of performing the intended use, then it meets the claim.

B) Applicant argues that Sakata et al. is at best ambiguous with regard to the effect of the reagent on erythrocytes.

In response, Sakata et al. makes clear his intent to permeabilize (but inhibit complete lysis of) leucocytes to allow permeation of fluorescent stain for incorporation into nucleic acids and to effectively and completely lyse erythrocytes so as not to interfere with detection of nucleated cells (leucocytes), since incomplete lysing of the erythrocytes resulting from improper nonionic surfactant dilution with ionic surfactants

causes for erythrocytes to remain in the treated sample as ghost cells (see column 9, line 50 to column 10, line 9). In as far as the statement of "the nonionic surfactant also will act to accelerate lysis of erythrocytes (which are intended to be lysed), which also causes a problem in the case of measuring leucocytes contained in the blood sample," the statement makes reference to the potential potent lysing activity by anionic surfactant which may unnecessarily damage the cell membrane of leucocytes, whose cell membranes are only intended to be permeabilized and not lysed. Accordingly, contrary to Applicant's argument, Sakata et al. intends for complete lysis of erythrocytes with effective permeabilization of leucocyte cell membrane.

C) Applicant argues that Sakata et al. in combination with Haas et al. and in light of Veriac et al. does not render obvious to the claimed invention because Sakata et al. can only selectively label leucocytes whereas the claimed invention also labels reticulocytes.

In response, the claimed invention recites a reagent for identifying and counting nucleated blood cells, which is consonant to the teaching of Sakata et al. Additionally, Applicant's argument is not on point and appears to be contradictory to the claimed invention because reticulocytes are not nucleated cells.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., the claimed reagent also labels reticulocytes) are not recited in the rejected claims. Although the claims are interpreted in light of the specification, limitations from the

specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

D) Applicant argues that there is no motivation to combine the teaching of Haas et al. with that of Sakata et al. to arrive to the claimed invention because Haas et al. is directed to use of ionophores with red blood cells and cells in tissue cultures and therefore, provides no insight into use of ionophores with leucocytes. Applicant further contends that the claimed invention does not seek to stain red blood cells or tissue culture cells because mature human red blood cells have no nuclei.

In response, while red blood cells are anucleated, tissue culture cells are nucleated; hence, are obvious equivalents or variations of nucleated cells as the leucocytes having nuclei, taught by Sakata, and for application in the method of Sakata et al.

E) Applicant argues that Veriac does not remedy the failed teaching of Sakata et al. and Haas et al. because while Sakata et al. does teach coloration of nucleated cells, Veriac does not teach coloration of nucleated cells.

In response, Haas et al. and Veriac are obviously combined with the teaching of Sakata et al. for the consonant motivation to effectively increase permeability of nucleated cells so as to promote penetration of molecules such as stain or flurorescent dye into the cell for incorporation with intracellular material, i.e. nuclei. Haas et al. specifically provide that valinomycin is an ionophore that is known, used, and

compatible with reagents and methods that employ potential-sensitive stains or dyes, and provides its advantageous use in circumventing permeability of cell membranes to specific charge sensitive molecules. Additionally, Applicant admits, by way of disclosure at page 4, lines 23-30, that inclusion of ionophores in a reagent solution assists in penetration of specific molecules into cells (selectively increases permeability of potential-sensitive molecules into cell membrane of blood cells as provided by Haas); such concept being taught and suggested early on in Applicant's French Patent no. 97 01090, dated 31 January 1997.

8. No claims are allowed.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (571) 272-0820. The examiner can normally be reached on Monday, Tuesday, and Thursday, 7:00 AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long V. Le can be reached on (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Gailene R. Gabel
Patent Examiner
Art Unit 1641
January ~~18~~ 22, 2007 grgabel